Figure 1A

SEQ ID NO:1

/translation="MGSVLSTDSGKSAPASATARALERRRDPELPVTSFDCAVCLEVL
HQPVRTRCGHVFCRSCIATSLKNNKWTCPYCRAYLPSEGVPATDVAKRMKSEYKNCAE
CDTLVCLSEMRAHIRTCQKYIDKYGPLQELEETAARCVCPFCQRELYEDSLLDHCITH
HRSERRPVFCPLCRLIPDENPSSFSGNLIRHLQVSHTLFYDDFIDFNIIEEALIRRVL
DRSLLEYVNHSNTT"

## Figure 1B

### SEQ ID NO:2

```
AGCGGAGGTCATTTTTGCAGCTTATTGTGATGACAACAGTGGAGGATGGTCTTCCACTTCA
CCTTAAAAGCGGCTGTTCTCTGATTATCATTAAGCATGGCCACGCCCGCACTTAACTTCTG
ACAGTGGGGAAAGCAGCTGTGTGTGATAGCTTGGAAGGTTTACTGCTGCCTCAAGTCCTCT
TCTCTGCAGTTGAGGTTTCAGGTTTCAATCCTCCCAATACCACAAGACAGAGCACGGGGCG
GCTGCCGCCTCCGCCTCCGCGCCTTAACCTAGGCGGCTTGCCGAAGATCTCAGCCCCGCGG
CCGCGCGCTCGCCCTGCCCTAGACCAGGGTTGGGCGCAGCGGCGGAGGTGGCTTCTGGGCT
GCGCGAGCTGGGAGAGCTGGGAGGCGGCGATCGCAGCTGGGCCGGGACTTCCTTCCTCCAC
CGCACGGCAACAAAACAACCCTGCGGCAGGCACTGAGTGCTTCGCAGCTGTCTGGGCGAGA
GGCACAGCGATGGGCTCCGTGCTGAGCACCGACAGCGGCAAATCGGCGCCCGCCTCTGCCA
CCGCGCGGGCCCTGGAGCGCAGGAGGGACCCGGAGTTGCCCGTCACGTCCTTCGACTGCGC
CGTGTGCCTTGAGGTGTTACACCAGCCTGTCCGGACCCGCTGCGGCCACGTATTCTGCCGT
TCCTGTATTGCTACCAGTCTGAAGAACAACAAGTGGACCTGTCCTTATTGCCGGGCATATC
TTCCTTCAGAAGGAGTTCCAGCAACTGATGTAGCCAAAAGAATGAAATCAGAGTATAAGAA
CTGCGCTGAGTGTGACACCCTGGTTTGCCTCAGTGAAATGAGGGCACATATTCGGACTTGT
CAGAAGTACATAGATAAGTATGGACCACTACAAGAACTTGAGGAGACAGCAGCAAGGTGTG
TATGTCCCTTTTGTCAGAGGGAACTGTATGAAGACAGCTTGCTGGATCATTGTATTACTCA
TCACAGATCGGAACGGAGGCCTGTGTTCTGTCCACTTTGCCGTTTAATACCCGATGAGAAT
CCAAGCAGCTTCAGCGGCAATTTAATAAGACATCTGCAAGTTAGTCACACTTTGTTTTATG
ATGATTTCATAGATTTTAATATAATTGAGGAAGCTCTTATCCGAAGAGTCTTAGACCGGTC
ACTTCTTGAATATGTGAATCACTCGAACACCACATAATTTTATTAAAACGAAGGGAAAAGG
GACCACTGAATTGCACCATTTAAGATGCTGCTTGAACAAATGGGAGGGAAGTTGTCAATGA
TTGATGGGCAAAAATGTACAACACAGTTATGTGTTTGTCCATGTTTATTGTTATAGTGCAT
TTAAAAACTGCTTTAATTTTAATGGTTTAAATCTGTTTTACATCCTTGAGATTCTTACACA
TCTAACAACAAAAAAAATTATCTACATCAGTCATTGTTACATGGAAAAGACAGGTGGTAGG
CAAGTAGGTGGAGGATCTCGGTTTGCAAATTAGATAATACTCTGTGTATAATGCTACATAT
CAATAACTACCATCATGGTTAGGCACGATAACTAATCTTTGTTCTGTGTAAAAAAATATGG
AGAGTGAAACAAAGTGCAGACATTCAAAGAAATAAGAAATCTGCTCCAATGCTCTTGTTCT
AATCTCTAATAGGTTAACGTTAATAATCTTGTATGGGAGTTGGAAAGGAAAATTTTGGAAG
TCAAGAAAGTCCATTTAGGCCGGACGCGGTGGCTTACGCTTGTAGTCCCAGCACTTTGGGA
GGCTGAGGCAGGCGGATCACAGGGTCGGGAGTTCGAGACCAGCCTGGCCAACACTGGTCTC
TGTGAAACTCCGTCTCTACTGAAAATGCAAAGATTGGCTGGACGTGTTGGCGGGCATCTGT
GATACCAGCTACTTGGGAGGCTGAGGCAGAAGAATCGCTTGAGCCCGGGAGGCGGAGGTTG
CAGTGAGCTGAGATCGCGCCAGTACACTCCAGCCTGGGTAACAGAGCTAGACTCCATCTCA
AAAAAAAAAAAAAAAAAAA
```

Figure 1C

SEQ ID NO:3

```
   1 acttctgaca gtggggaaag cagctgtgtg tgatagcttg gaaggtttac tgctgcctca
  61 agtcctcttc tctgcagttg aggtttcagg tttcaatcct cccaatacca caagacagag
 121 cacggggcgg ctgccgcctc cgcctccgcg ccttaaccta ggcggcttgc cgaagatctc
 181 agccccgcgg ccgcgcgctc gccctgccct agaccagggt tgggcgcagc ggcggaggtg
 241 gcttctgggc tgcgcgagct gggagagctg ggaggcggcg atcgcagctg ggccgggact
 301 tccttcctcc accgcacggc aacaaaacaa ccctgcggca ggcactgagt gcttcgcagc
 361 tgtctgggcg agaggcacag cgatgggctc cgtgctgagc accgacagcg gcaaatcggc
 421 gcccgcctct gccaccgcgc gggccctgga gcgcaggagg acccggagt tgcccgtcac
 481 gtccttcgac tgcgccgtgt gccttgaggt gttacaccag cctgtccgga cccgctgcgg
 541 ccacgtattc tgccgttcct gtattgctac cagtctgaag aacaacaagt ggacctgtcc
 601 ttattgccgg gcatatcttc cttcagaagg agttccagca actgatgtag ccaaaagaat
 661 gaaatcagag tataagaact gcgctgagtg tgacaccctg gtttgcctca gtgaaatgag
 721 ggcacatatt cggacttgtc agaagtacat agataagtat ggaccactac aagaacttga
 781 ggagacagca gcaaggtgtg tatgtccctt ttgtcagagg gaactgtatg aagacagctt
 841 gctggatcat tgtattactc atcacagatc ggaacggagg cctgtgttct gtccactttg
 901 ccgtttaata cccgatgaga atccaagcag cttcagcggc aatttaataa gacatctgca
 961 agttagtcac actttgtttt atgatgattt catagatttt aatataattg aggaagctct
1021 tatccgaaga gtcttagacc ggtcacttct tgaatatgtg aatcactcga acaccacata
1081 attttattaa aacgaaggga aaagggacca ctgaattgca ccatttaaga tgctgcttga
1141 acaaatggga gggaagttgt caatgattga tgggcaaaaa tgtacaacac agttatgtgt
1201 ttgtccatgt ttattgttat agtgcattta aaaactgctt taatttttaat ggtttaaatc
1261 tgttttacat ccttgagatt cttacacatc taacaacaaa aaaaattatc tacatcagtc
1321 attgttacat ggaaaagaca ggtggtaggc aagtaggtgg aggatctcgg tttgcaaatt
1381 agataatact ctgtgtataa tgctacatat caataactac catcatggtt aggcacgata
1441 actaatcttt gttctgtgta aaaaaatatg gagagtgaaa caaagtgcag acattcaaag
1501 aaataagaaa tctgctccaa tgctcttgtt ctaatctcta ataggttaac gttaataatc
1561 ttgtatggga gttggaaagg aaaattttgg aagtcaagaa agtccattta ggccggacgc
1621 ggtggcttac gcttgtagtc ccagcacttt gggaggctga ggcaggcgga tcacagggtc
1681 gggagttcga gaccagcctg gccaacactg gtctctgtga aactccgtct ctactgaaaa
1741 tgcaaagatt ggctggacgt gttggcgggc atctgtgata ccagctactt gggaggctga
1801 ggcagaagaa tcgcttgagc ccgggaggcg gaggttgcag tgagctgaga tcgcgccagt
1861 acactccagc ctgggtaaca gagctagact ccatctcaaa aaaaaaaaaa aaaaaa
```

Figure 1D


SEQ ID NO:4


```
127        gcgg ctgccgcctc cgcctccgcg ccttaaccta ggcggcttgc cgaagatctc
181 agccccgcgg ccgcgcgctc gccctgccct agaccagggt tgggcgcagc ggcggaggtg
241 gcttctgggc tgcgcgagct gggagagctg ggaggcggcg atcgcagctg ggccgggact
301 tccttcctcc accgcacggc aacaaaacaa ccctgcggca ggcactgagt gcttcgcagc
361 tgtctgggcg agaggcacag cgatgggctc cgtgctgagc accgacagcg gcaaatcggc
421 gcccgcctct gccaccgcgc gggccctgga gcgcaggagg gacccggagt tgcccgtcac
481 gtccttcgac tgcgccgtgt gccttgaggt gttacaccag cctgtccgga cccgctgcgg
541 ccacgtattc tgccgttcct gtattgctac cagtctgaag aacaacaagt ggacctgtcc
601 ttattgccgg gcatatcttc cttcagaagg agttccagca actgatgtag ccaaaagaat
661 gaaatcagag tataagaact gcgctgagtg tgacaccctg gtttgcctca gtgaaatgag
721 ggcacatatt cggacttgtc agaagtacat agataagtat ggaccactac aagaacttga
781 ggagacagca gcaaggtgtg tatgtccctt ttgtcagagg gaactgtatg aagacagctt
841 gctggatcat tgtattactc atcacagatc ggaacggagg cctgtgttct g
```

Figure 1E

SEQ ID NO:5

TRAC1 genomic region:

```
acttttagttgaatttctgattcctgccatcaaaagtaagtcttgcaggaattttttagata
atattaaggatttggaatttaatcctaaggattaggagagctattaaaggattttgtgcat
ggggtgacacaagatgtttgcttttcaaaagatcactttagttgccatgtggataataaac
tggagagaggcaatgatggatgcgggtagagcagttaggaactactgccattaagtcacac
aagagaatgcagtgatttaaaataagcggtggctatggaaatagaagaaaatgagcatggt
ggctcacgcctgtaatcccagcactttgggaggccaaggcaggcggatcatgaggtcagga
gttcacgactagcctggccaacatggtgaaactccgtctctaccaaaaatataataaaatt
agccgggtgtggtggcatgcgcctgcaatcccagctactcaggaggctgaggcaggagaat
cacttgaacccaggaggcagaggttgcagtgagctgagattgcgccactgcacacttccag
cctgggcgacagagagagacttcatctcagaaaaaaaagaactactgagatacatattgga
ggcaggattgtgattatgcttgattcaatgtgaatgaggaagaggaagaaatcaaggatga
cttccaggtgtctagactgagctataaagtggatcatagtgccatttgctaaaagagagat
caaccactggaggaggctgctactataatgagttcattattggagacattgggctgagggt
gtttatgtcaaatggtcagtcatgtaagctattaggctattggacatttgaatggcctggg
gtagagataaagatgtgaaagtttttggaaccgaaatagtgacagaacctgtgtgatttttt
gagagaaagagaaagagagaaagagagagagaggagaagggaaaaggtcctaagacagaat
cctaggatctccagcccagagccagtggagaaggagactctggcaagggagaagaaacaat
agccagaaagaaagagggaaagtcagagtgtttccagaaggaggggatggtcagcactaac
aaacatagttgaggggtcaagcaaaaaaaatagctgaaaagaatctattggaattagttac
atgaacgtcaccagtgacactgataataaagcagttttggacagatggaggtggagaggt
tggttcagaacccagactggactgaataagaagtgaataattaagaaatgatgacaaaatg
tagaggatcagatcaagagatttggccttgaagcggatatggggcaggagttgaggcataa
gtgggatgaaagggaggttttgttttccttttgaagatgggaataactacctttttcatct
tatttccccccccccaccgcctgcccccaccacccatgctccagctccataggtctttcc
tttctttggttctgcctcattcactgttcccagtccacgctttttcctaaatctttgaatg
gctcacttcttttcattattcatggctctgctcaaatgtcacttcatccaaaaattcttct
ctgagcttattcttctcctcattgccatttactgttttatcttcttcatggctctgattat
ctgaaatgattttgttcatttgtttatggattgattacatgtatcttccttttgtttgttg
tttgttttttgagacagagtctcgctctgttacccacgatggagtgtagtggcatggtctcg
gctcactgcaacctccacctcttgggtgcaagtggttctcctgcctcagcctcccaagtag
ctgggactacacgcatgtgccaccacacctggctaattttttgtatttttagtacaaaaatt
tttactaaagaaatgtatttctagtagagacggggtttcactatgttggccaggctggtct
tgaactcctgaccttgtgatccacctgcctcggcctcccaaattgctgggatttcaggcgt
gagccactgcacccagcctgttttggttttttgagacagggtctccctctgttgcccaggc
tggagtgcagtggcatgatctcggctcactgcagcctccacctttccggttcaagcgattc
ttgtgcctcagcctcccaagtagctcacacccagctaagttttgtattttttagtagagaca
gggtttcaccatgttggccaggctggtctcaaactcctgacctcaggtgatctgtccttct
tgcctcccaaagtgctgggattacaggcgtgagccactgccccggcttggtttgttttttg
agacatggtctcactctgtcgcacaggacggagtgcagtagtgtgatcttggctcccgtgc
tcaagtgatcctcccacctcagcctcccaaagtgctgaaattacaggcgtgagccacggta
cccggcctatatctcctaattaaaagtaaagcctgggtgaagtttatggcctgctaaatac
```

gtacatgtattatatgcgttattatatcccatgtgttatattatatattattgtatatgtg
tattatatactatgtactattcttacaactgtatgtgaatctataattatctcaaagtttt
gttttgtttttaaagtaagttctgtgagaactgggatttgtttgcttgttgacgattg
tgtcttcatcactagaaccgagtctggcgtgcagtgggcactaaataagtcttcgtaaaaa
gtgtaaattaacgcccggctaattttgtattattagcagcgacggggtttcaccatgttg
gccaggctggtctcgaactcctgccctcaagcaggagtttcaagcccgcctcggcctccca
aagtgttggaattacaggcgtgggccaccgcgcctggctgatcatgtttaaagggcggggt
ggggagcgtacaaacaacagaggaaatcctgagccgcagaggaaactggagatggcagggt
ttagcactagaatctctgtaggagaggtaagattggaatcctaatctccaggactcttttcc
actacccaggctatctctccattaatggactattgattggatgacagagtagcgatagcac
cgtcttaggagacgcccaatcataggtcataggtcatttttgcagcttattgtgatgacaa
cagtggaggatggtcttccacttcaccttaaaagcggctgttctctgattatcattaagca
tggccacgcccgcactta
ACTTCTGACAGTGGGGAAAGCAGCTGTGTGTGATAGCTTGGAAGGTTTACTGCTGCCTCAA
GTCCTCTTCTCTGCAGTTGAGGTTTCAGGTTTCAATCCTCCCAATACCACAAGACAGAGCA
CGGGGCGGCTGCCGCCTCCGCCTCCGCGCCTTAACCTAGGCGGCTTGCCGAAGATCTCAGC
CCCGCGGCCGCGCGCTCGCCCTGCCCTAGACCAGGGTTGGGCGCAGCGGCGGAGGTGGCTT
CTGGGCTGCGCGAGCTGGGAGAGCTGGGAGGCGGCGATCGCAGCTGGGCCGGGACTTCCTT
CCTCCACCGCACGGCAACAAAACAACCCTGCGGCAGGCACTGAGTGCTTCGCAGCTGTCTG
GGCGAGAGGCACAGCGATGGGCTCCGTGCTGAGCACCGACAGCGGCAAATCGGCGCCCGCC
TCTGCCACCGCGCGGGCCCTGGAGCGCAGGAGGGACCCGGAGTTGCCCGTCACGTCCTTCG
ACTGCGCCGTGTGCCTTGAGGTGTTACACCAGCCTGTCCGGACCCGCTGCGGCCACGTATT
CTGCCGTTCCTGTATTGCTACCAGTCTAAAGAACAACAAGTGGACCTGTCCTTATTGCCGG
GCATATCTTCCTTCAGAAGGAGTTCCAGCAACTGATGTAGCCAAAAGAATGAAATCAGAGT
ATAAGAACTGCGCTGAGTGTGACACCCTGGTTTGCCTCAGTGAAATGAGGGCACATATTCG
GACTTGTCAGAAGTACATAGATAAGTATGGACCACTACAAGAACTTGAGGAGACAGCAGCA
AGGTGTGTATGTCCCTTTTGTCAGAGGGAACTGTATGAAGACAGCTTGCTGGATCATTGTA
TTACTCATCACAGATCGGAACGGAGGCCTGTGTTCTGTCCACTTTGCCGTTAATACCCGA
TGAGAATCCAAGCAGCTTCAGTGGCAGTTTAATAAGACATCTGCAAGTTAGTCACACTTTG
TTTTATGATGATTTCATAGATTTTAATATAATTGAGGAAGCTCTTATCCGAAGAGTCTTAG
ACCGGTCACTTCTTGAATATGTGAATCACTCGAACACCACATAATTTTATTAAAACGAAGG
GAAAAGGGACCACTGAATTGCACCATTTAAGATGCTGCTTGAACAAATGGGAGGGAAGTTG
TCAATGATTGATGGGCAAAAATGTACAACACAGTTATGTGTTTGTCCATGTTTATTGTTAT
AGTGCATTTAAAAACTGCTTTAATTTTAATGGTTTAAATCTGTTTTACATCCTTGAGATTC
TTACACATCTAACAACAAAAAAAATTATCTACATCAGTCATTGTTACATGGAAAAGACAGG
TGGTAGGCAAGTAGGTGGAGGATCTCGGTTTGCAAATTAGATAATACTCTGTGTATAATGC
TACATATCAATAACTACCATCATGGTTAGGCACGATAACTAATCTTTGTTCTGTGTAAAAA
AATATGGAGAGTGAAACAAAGTGCAGACATTCAAAGAAATAAGAAATCTGCTCCAATGCTC
TTGTTCTAATCTCTAATAGGTTAACGTTAATAATCTTGTATGGGAGTTGGAAAGGAAAATT
TTGGAAGTCAAGAAAGTCCATTTAGGCCGGACGCGGTGGCTTACGCTTGTAATCCCAGCAC
TTTGGGAGGCTGAAGCAGGCGGATCACAAGGTCAGGAGTTCGAGACCAGCCTGGCCAACAC
TGGTCTCTGTGAAACTCCGTCTCT

## Figure 1F

SEQ ID NO:6
Mouse TRAC1 cDNA sequence:

```
TGCGCGCGCTCCGCCTGCGCTCCACCGAGAGGCCCCGCGGCGCGGCTGGC
CGAGCCAGGAGGCGGCGATCCGGCCTGGGCCGGGACTTCCTTCCTCCCGG
GCGGGACAACAGAACCACCCGCAGCCAGCAGGGAGCTCCTGGCAGCTCGC
TGAGCTAGAGCGCAAGNNTGGGCTCCCTGCTGAGCAGCGACAGCTCCAAG
TCCGCGCCCGCCTCCGCCACCCCGCGGACTCTGGAGCGCAGCGGGGACTC
GGAGCTGCCCATCACCTCCTTCGACTGCTCAGTGTGTCTGGAGGTGCTAC
ACCAGCCGGTCCGGACCCGCTGTGGCCACGTGTTCTGCCGATCTTGCATT
GCGACCAGTATAAAGAACAATAATAAATGGACATGTCCATACTGCCGGGC
ATACCTTCCTTCAGAGGGAGTGCCCGCAACTGACATAGCCAAGAGGATGA
AGTCAGAATACCAGAACTGTGCTGAGTGTGGAACTCTGGTTTGCCTCAGT
GACATGAGGGCGCACATAAGGACCTGTGAGAAGTACATCGATAAATATGG
CCCGCTGCTAGAACTTGGCGACACCACAGCAAGATGTGTATGTCCATTTT
GTCAGCGGGAACTGGATGAAGACTGCTTGCTGGATCATTGCATTATCCAC
CACAGATCAGAAAGGAGGCCCGTGTTCTGTCCACTTTGCCATTCACGACC
TGATGAAAGCCCAAGTACCTTCAATGGCAGTTTAATTAGACATTTGCAAG
TCAGTCACACTTTGTTTTATGATGATTTCATAGATTTTGATATAATTGAG
GAAGCCATTATTCGCAGAGTGCTAGACCGCTCACTTCTTGAATATGTGAA
TCAGTCAAACACCACATAATTTTATGACTAGGAAGGGGACCATTCACTCG
TACCATTTAAGATGCTGCTTGAAGGACTGGAGGGGGATTGTCGACGTTTG
ATGGAGAAACATGTACTACAGTATTACCTGCTTGTCTTTGTTATGTTGCA
TTCAAAAACCGTGTTCATTCTGGCTTAGATCTGCCCTTACATTCTTGAGT
CTATTAGACATTTAACCACGAGACACAGCCTCTCAGCCATTAACAGATGG
AAGAGACACGACACAGGCTGGCTATGTGATGGAGCAGCTTCCTGTGTCCA
TTAGTGATGTGTGTATAACGCTACATATTCCCAACTGTCATCATGGTTAG
GCGAGAAAGCCAATCTCTGTTCCATGCTAAAAGACGGAGAAAGGAACAAA
ATATGGACATTAARGGAAATCTGAAATGTATTCAAATTCTCATGCTCTAA
TCTCCAATAAGGAACGTGAATAATCTTATGAAAAGGAGGAAAGGGAAGAA
TTTGAAGTCACCAAAATCCAATTTAGCCAAATTATAGTACATAATATAAT
ACTGCAGC
```

SEQ ID NO:7
Mouse TRAC1 protein (3$^{rd}$ frame)

```
SAXXGSLLSSDSSKSAPASATPRTLERSGDSELPITSFDCSVCLEVLHQP
VRTRCGHVFCRSCIATSIKNNNKWTCPYCRAYLPSEGVPATDIAKRMKSE
YQNCAECGTLVCLSDMRAHIRTCEKYIDKYGPLLELGDTTARCVCPFCQR
ELDEDCLLDHCIIHHRSERRPVFCPLCHSRPDESPSTFNGSLIRHLQVSH
TLFYDDFIDFDIIEEAIIRRVLDRSLLEYVNQSNTTFYD
```

# FLJ20456 Hit Inhibited anti-TCR Induced CD69 Expression in Jurkat Cells
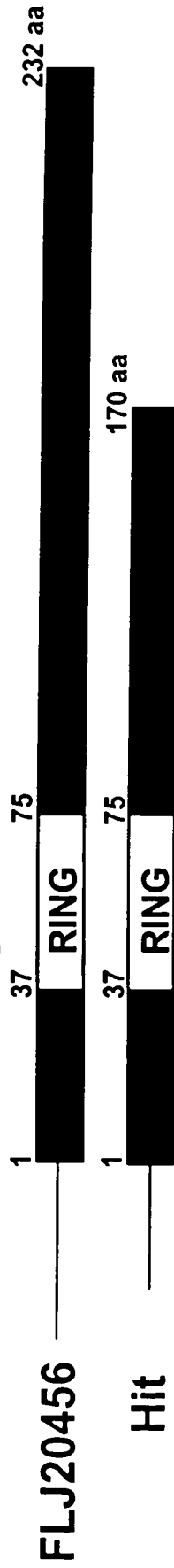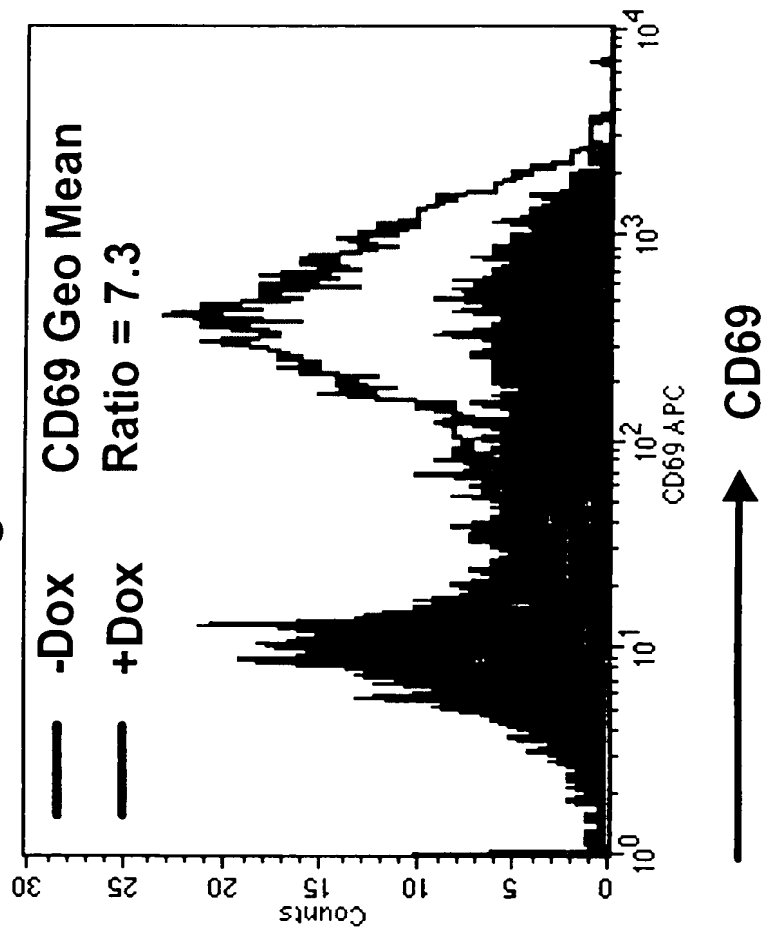
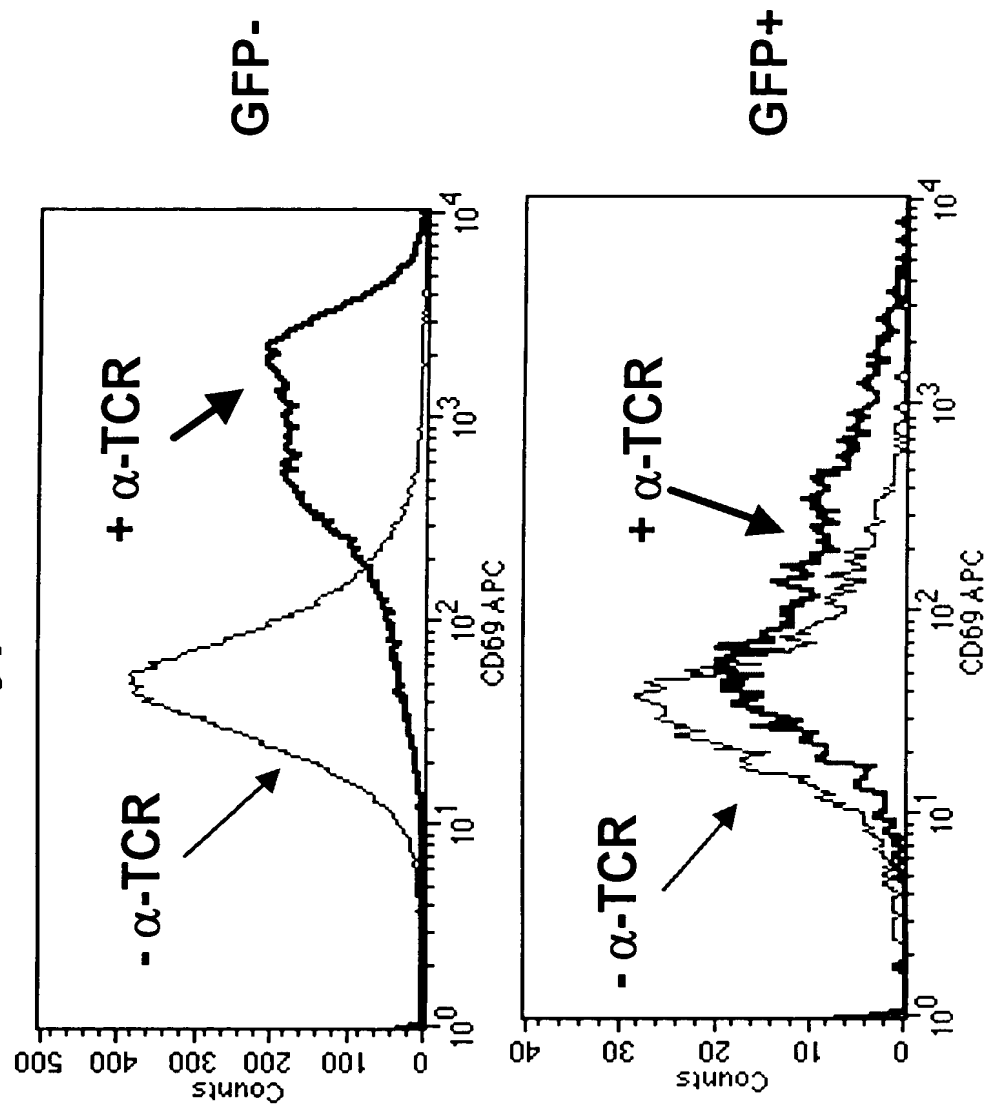# FLJ20456 Hit Inhibited anti-TCR Induced CD69 Expression in Jurkat Cells Phenotype Transfer

# Full Length FLJ20456 Does Not Inhibit CD69 Upregulation in Jurkat Cells

**FLJ20456**

1    37    75    232 aa

RING

- Pfu PCR product amplified from a capped human brain cDNA library.
- One N to S polymorphism with FLJ20456 NM_017831.1 at amino acid 186, present in EST database.
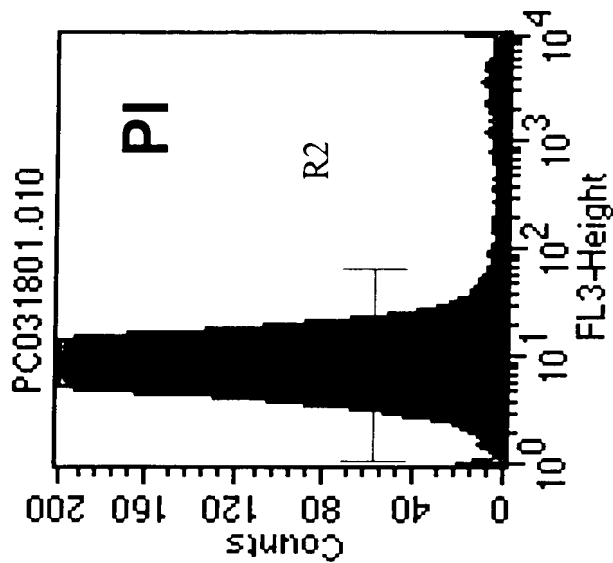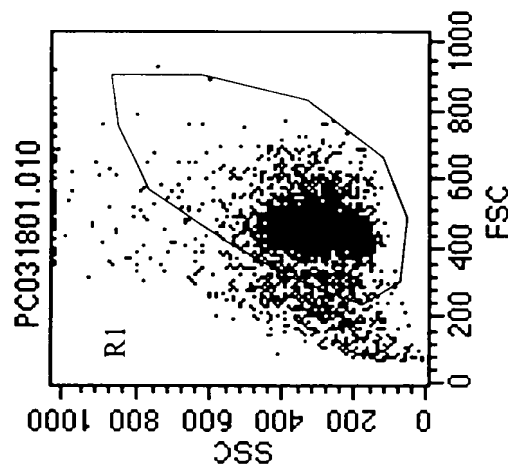
**JurkatN 32H**

PC031801.010

PI

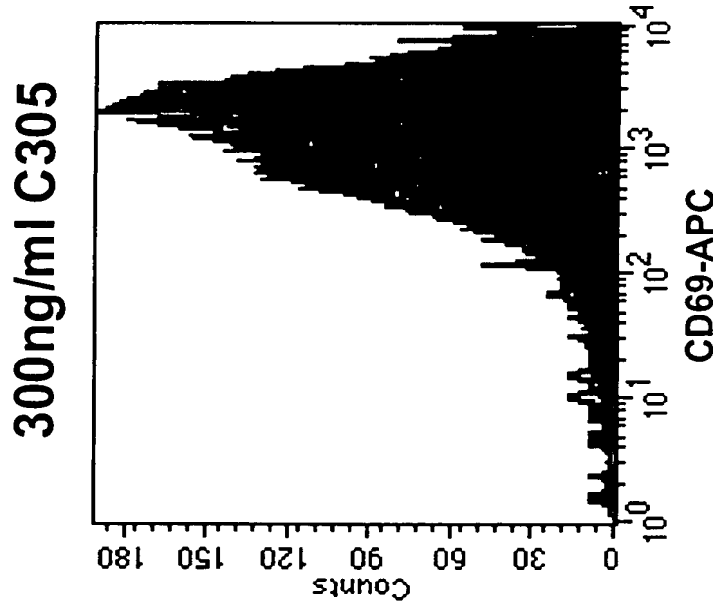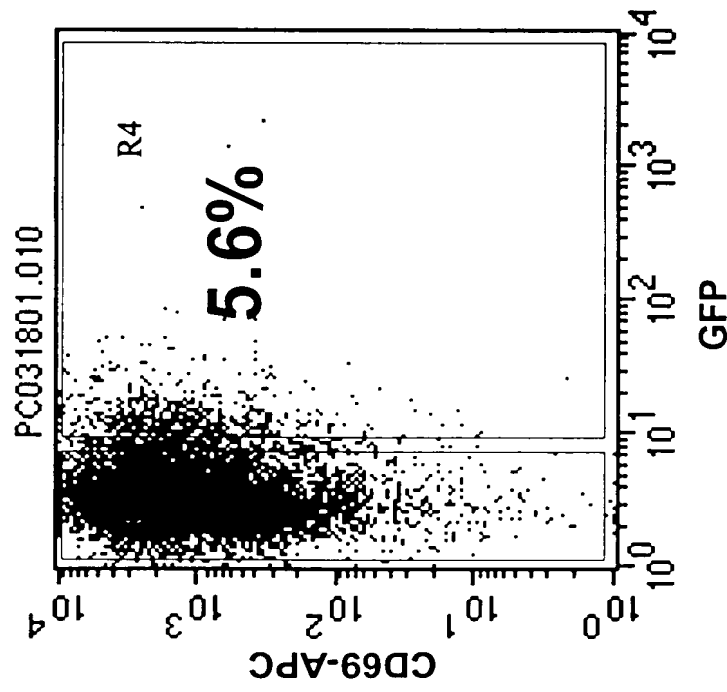R2

PC031801.010

R1

Figure 3B

# Full Length FLJ20456 Does Not Inhibit CD69 Upregulation in Jurkat Cells

GFP-    1070.5

GFP+   1219.9

Ratio =  0.88

Figure 4A

FLJ20456 Hit Specifically Inhibited T Cell Activation but not B Cell Activation
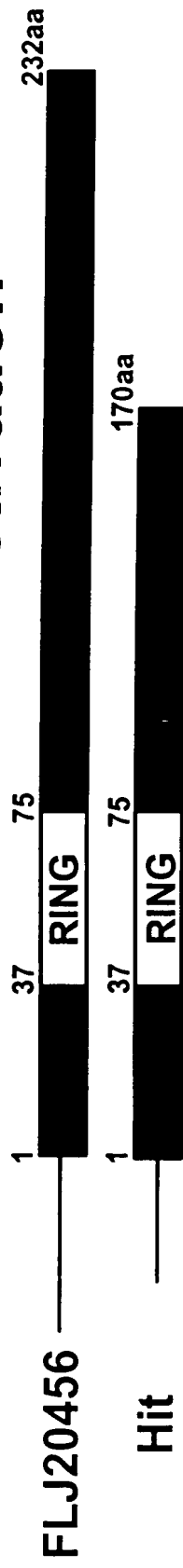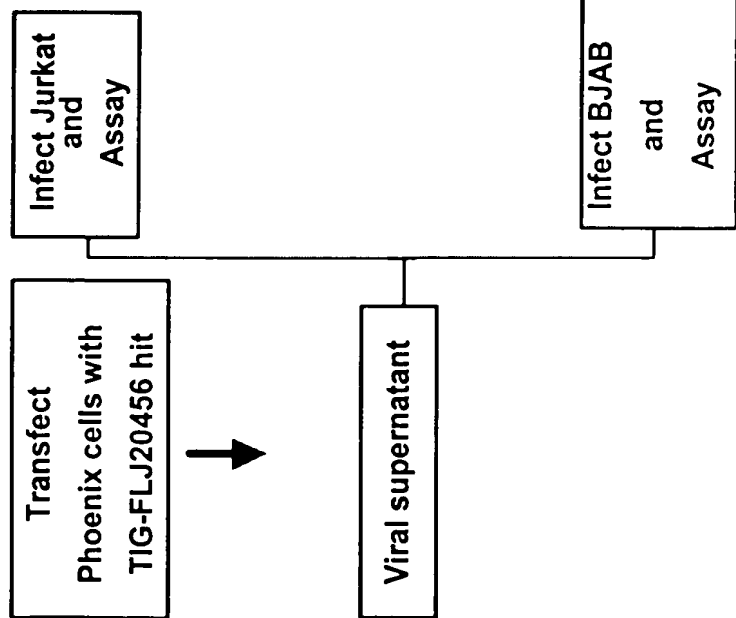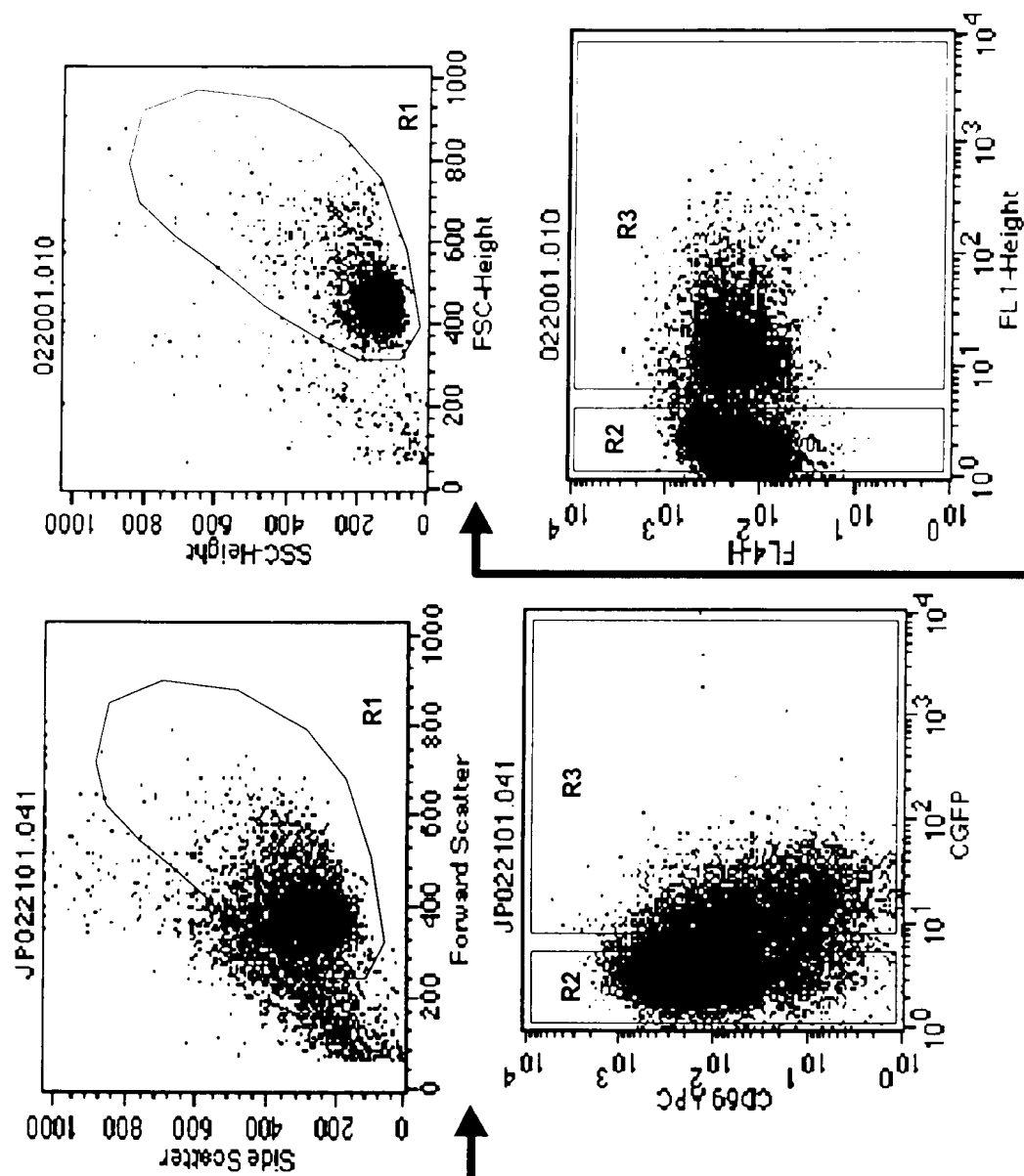
Figure 4B

Figure 4C

**Jurkat**
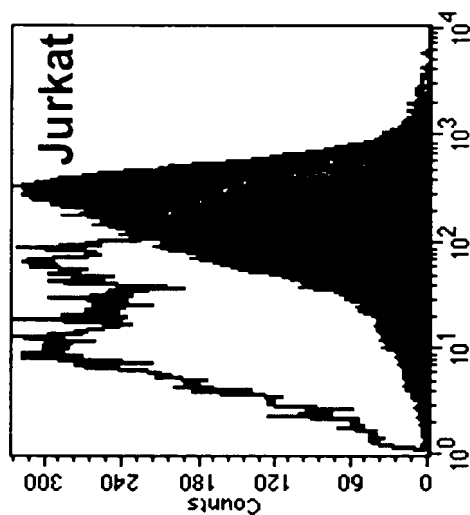
Stimulation:
300ng/ml C305

GFP-  141.2
GFP+  28.8

Ratio = 4.9

Counts (y-axis: 0, 60, 120, 180, 240, 300)

CD69-APC ($10^0$, $10^1$, $10^2$, $10^3$, $10^4$)

**BJAB**
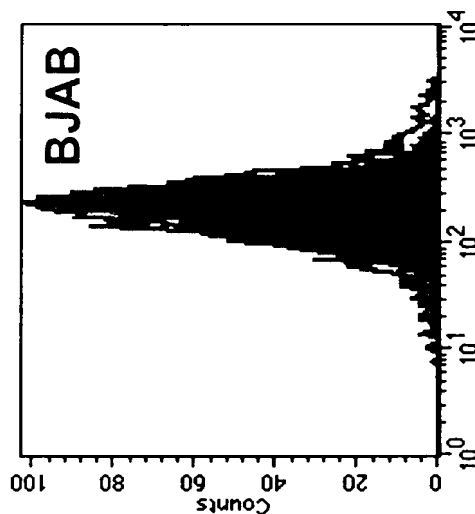
Stimulation:
300ng/ml α-IgM

GFP-  183.1
GFP+  183.3

Ratio = 1.0

Counts (y-axis: 0, 20, 40, 60, 80, 100)

CD69-APC ($10^0$, $10^1$, $10^2$, $10^3$, $10^4$)

Figure 5

# FLJ20456 Is Strongly Expressed in Lymphoid and Hematopoietic Organs

Probe: FLJ20456 Hit



- FLJ20456 is expressed in multiple tissues
- The strongest expression is in PBL, Liver and Spleen
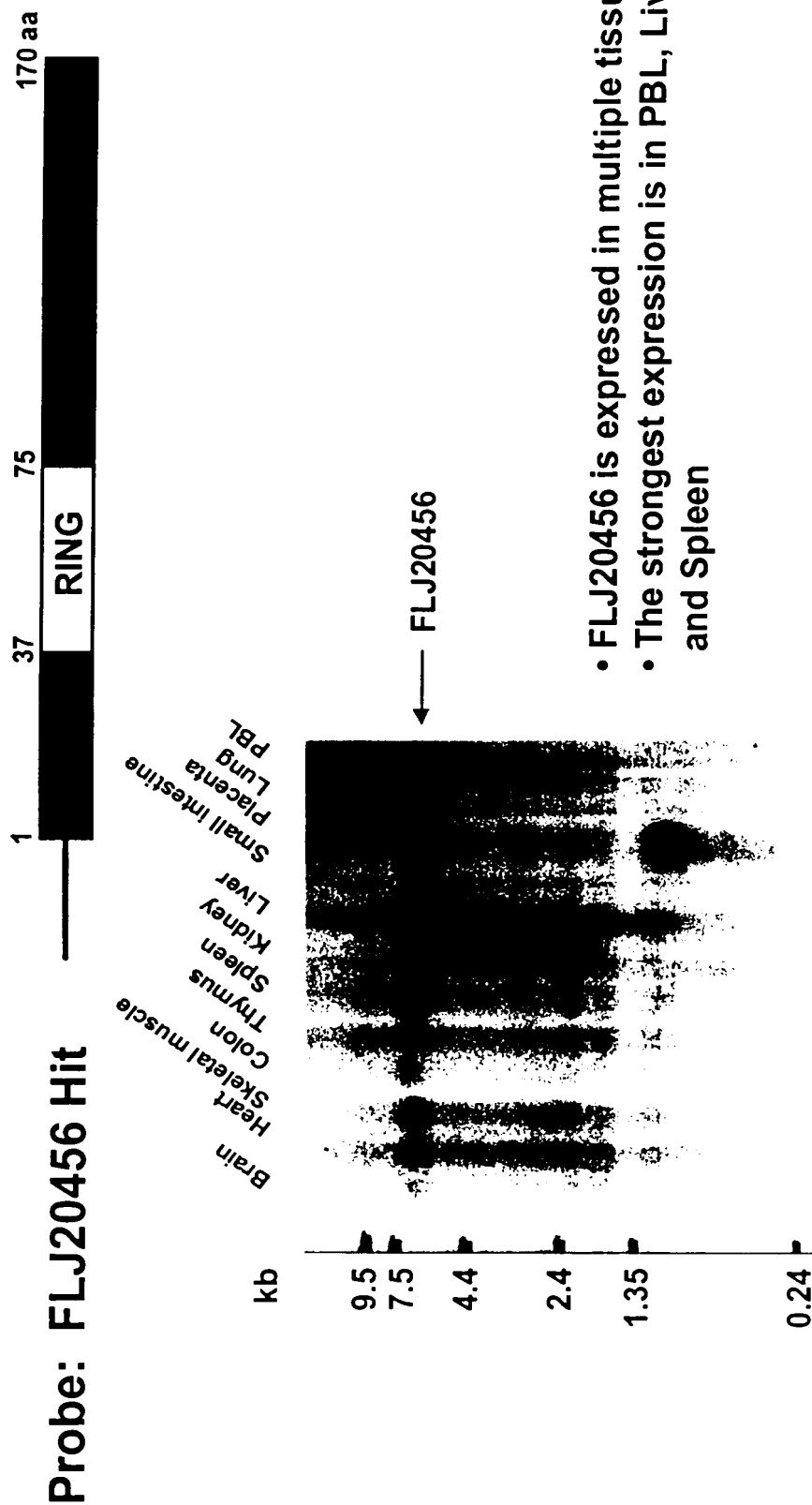
Figure 6

# TRAC1 is Predominantly Expressed in Resting T lymphocytes

Relative expression

Primer/Probe SetA

■ TRAC1/HuPO

| Sample | Value |
|--------|-------|
| reCD14 | 5.2 |
| act CD19 | 0.4 |
| re CD19 | 1.5 |
| act CD8 | 2.9 |
| re CD8 | 17.4 |
| act CD4 | 2.4 |
| re CD4 | 12.4 |
| act PBMC | 3.5 |
| PBMC | 5.1 |
| Fetal Sk. Mus. | 2.5 |
| Fetal Thymus | 2.6 |
| Fetal Spleen | 1.6 |
| Fetal Heart | 0.6 |
| Fetal Kidney | 0.4 |
| Fetal Liver | 0.3 |
| Fetal Lung | 0.5 |
| Fetal Brain | 0.1 |
| Tonsil | 0.8 |
| Fetal Liver | 0.3 |
| Bone Marrow | 0.7 |
| PBL | 6.6 |
| Thymus | 1.7 |
| Lymph Node | 2.3 |
| Spleen | 2.7 |
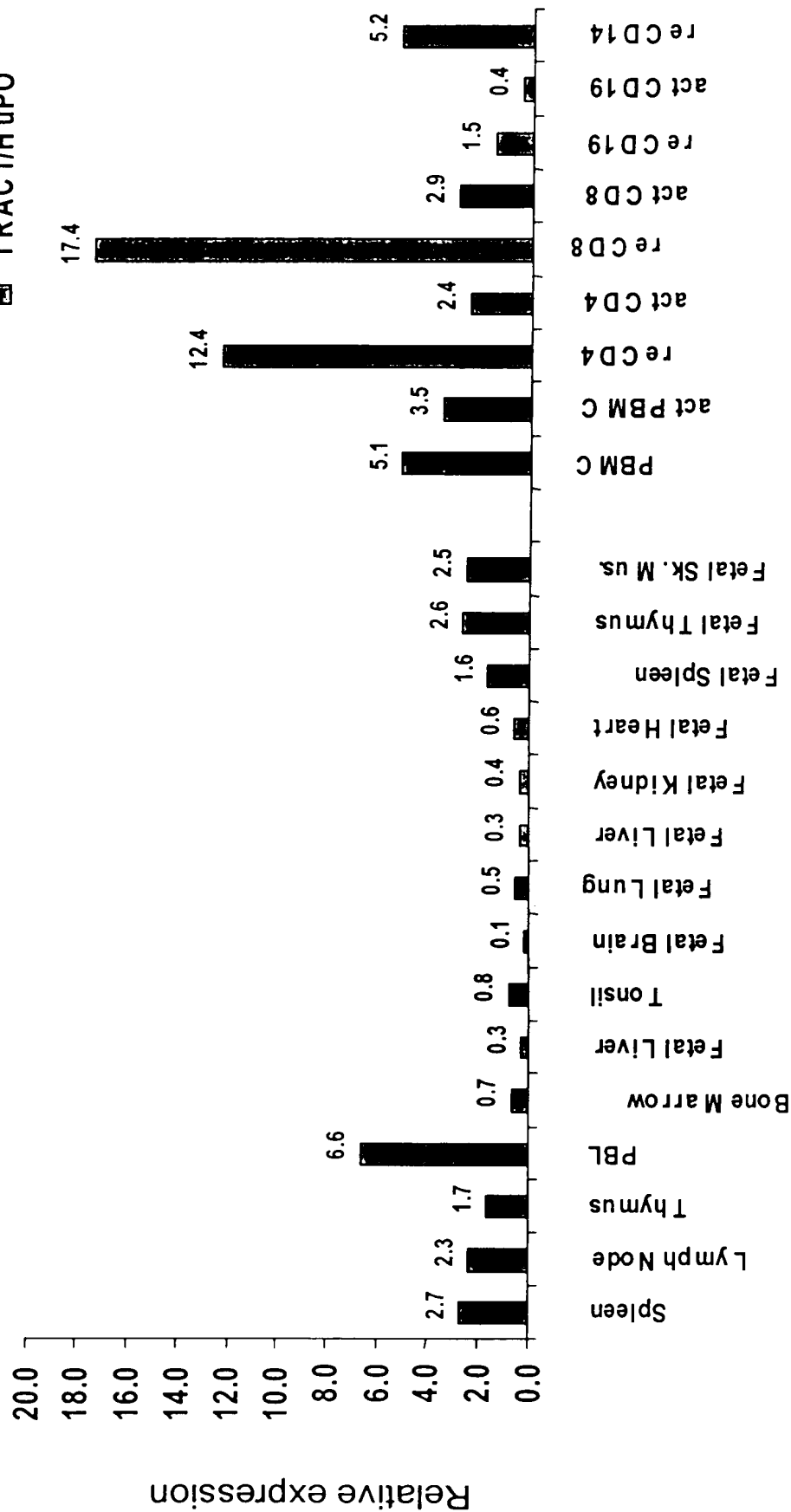
# FLJ20456 Sequence is Most Similar to Two Sequences: Znf313 and STRIN

Ring Domain

```
Consensus   #1  ....................................F.C.VC.EV...PV....C.HVFC...C.........

FLJ20456.pep  MSSVLSTDSGKSAPASATARALERRRDPELPVTSFDCAVCLEVHQPVR-DRCGHVFCRSCIADSLRN  67
znf313.pep    -----MAAQQRDCGGAAQLAGPAAEADPLGRFTCPVCLEVYEKPVQV-PCGHVFCSACLQECLKP   59
STRIN.pep     ---------MAEDLSAATSYTEDDEYCPVCQEVIKTPVRTTACQHVFCRKCFLTAMRE         49

Consensus   #1  ......C..CR........................A.........C..C.........R.H...C.KY...Y......

FLJ20456.pep  NKWTCPICRAYLPSE----GVPATDVAKRMKSEYKNCAECDTLVCLSEMRAHIRTCQKYIDKYGPLQE  131
znf313.pep    KPVCGVCRSALAP-----GVRAVELERQIESTETSCHGCRKNFFLSKIRSHVATCSKYQN-YIMEGV  121
STRIN.pep     SGAHCPLCRGNVTRRERACPERAIDLENIMRKFSGSCRQCAKQIKFYRMHYKSCKKYQDEYGVSSI  117

Consensus   #1  .........................CP.C..........L..HC...H..............

FLJ20456.pep  LEETAAR------------------------------------CVCPFCQRELYE-DSLLDHCITHRSERR  166
znf313.pep    KATIKDASLQPRNVPNRY--------------------------TFPCPYCPKNFDQEGLVEHCKLFHSDTK  169
STRIN.pep     VPNFQISQDSVGNSNRSETSTSDNTETYQENTSSSGHPTFKCPLCQESNFTRQRLLDHCNSNHLFQIV  185

Consensus   #1  .V.CP.C...P...P....N..H......Y.F....EE............S.............

FLJ20456.pep  PMFCPLCRLIPDENPSSFSGNLIRHLQVSHTLFYDDFIDFNIIEEALIRRVLDRSLLEYVNHSNTT.  233
znf313.pep    SMVCPICASMPWGDPNYRSANFREHIIQRHRHFSYDTFVDYDVDEEDMMNQVLQRSIIDQ.       229
STRIN.pep     PMTCPICVSHPWGDPSQITRNEVSHLNQRQEDYGEEMNLQIDEETQYQTAVEESFQVNI.        246
```



| | 1 | 2 | 3 | |
|---|---|---|---|---|
| 1 | | 26.6 | 22.3 | 1 | FLJ20456.pep |
| 2 | 130.4 | | 27.9 | 2 | znf313.pep |
| 3 | 140.9 | 134.7 | | 3 | STRIN.pep |
| | 1 | 2 | 3 | |

* All three sequences are human
* Murine sequences are not shown

*FIG. 7.*

Alignment of RING Domain Sequences from Various Human Proteins

```
Consensus #1   .....C..C.........C.H..C.........C..........

FLJ20456.Ring  VTSFDCAVCLEVLHQPVRTR-CGHVFCRSCIATSL--KNNKWTCPYCRAYLP-S  50
znf313.Ring    LGRFTCPVCLEVYEKPVQVP-CGHVFCSACLQECL--KPKKPVGVCRSALA-P   50
STRIN.Ring     EDDFYCPVCQEVLKTPVRTACQHVFCRKCFLTAM--RESGAHCPLCRGNV--T   50
TRAF6.Ring     ESKYECPICLMALREAVQTP-CGHRECKACIIKSI--RDAGHKCPV-DNEILLE  50
c-Cbl.Ring     STFQLCKICAEN-DKDVKIEPGGHLMCTSCLTSW--QESEGQGCPFRCEI-KG   50
BRCA1.Ring     ---LECPICLELIKEPVSTK-CDHIFCKFCMLKLLNQKKGPSQCPLCKNDITKR. 50
BAR.Ring       VSEFSCHCCYDIIVNPT--TLNCGHSFCRHCLALWWA-SSKKTECPECREK--WE 50
RAG1.Ring      VKSISCQICEHILADPVETN-CKHVFCRVCILRCL--KVMGSYCPSCRYPCF-P  50
```

RING

FLJ20456

TRAF6    MATH

Cbl    UB A

BRCA1    BRCT  BRCT

BARD1    SAM

RAG1

FIG. 8.

Figure 9

# RING finger vs. Zinc finger proteins

**Ring-HC:** $C_3HC_4$ = Cys in position 5
**Ring H2:** $C_3H2C_3$ = His in position 5

- Ring finger domains have a conserved pattern of Cys and His residues that co-ordinate two zinc atoms to form a cross-brace structure
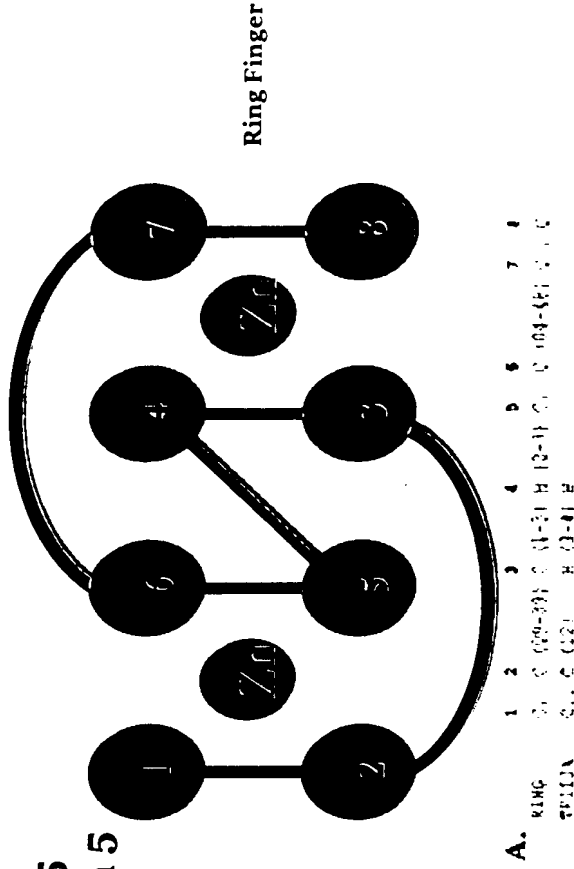
- Ring fingers are structurally distinct from zinc fingers



Ring Finger
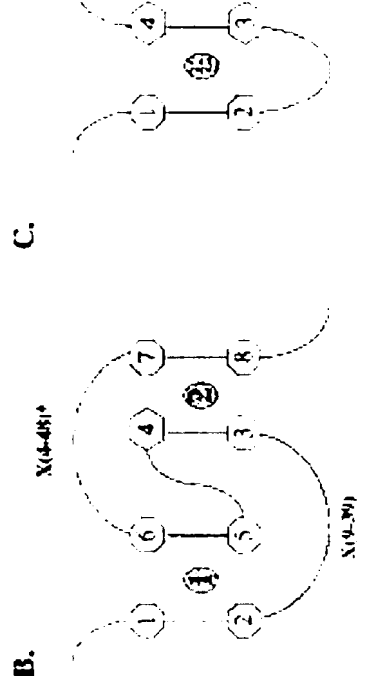
Zinc Finger

# Ubiquitin Pathway Components

- E1: ubiquitin-activating enzyme, with a major isoform that may work broadly

- E2: ubiquitin-conjugating enzyme, a class of ~14 enzymes, interacts with E3

- E3: ubiquitin ligases, a broad and growing group of activities that promote addition of ubiquitin to specific proteins

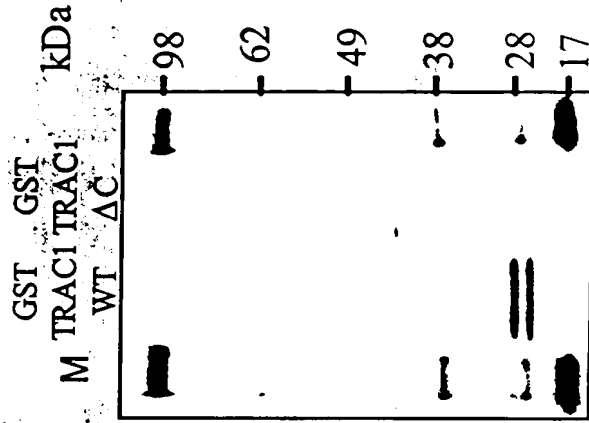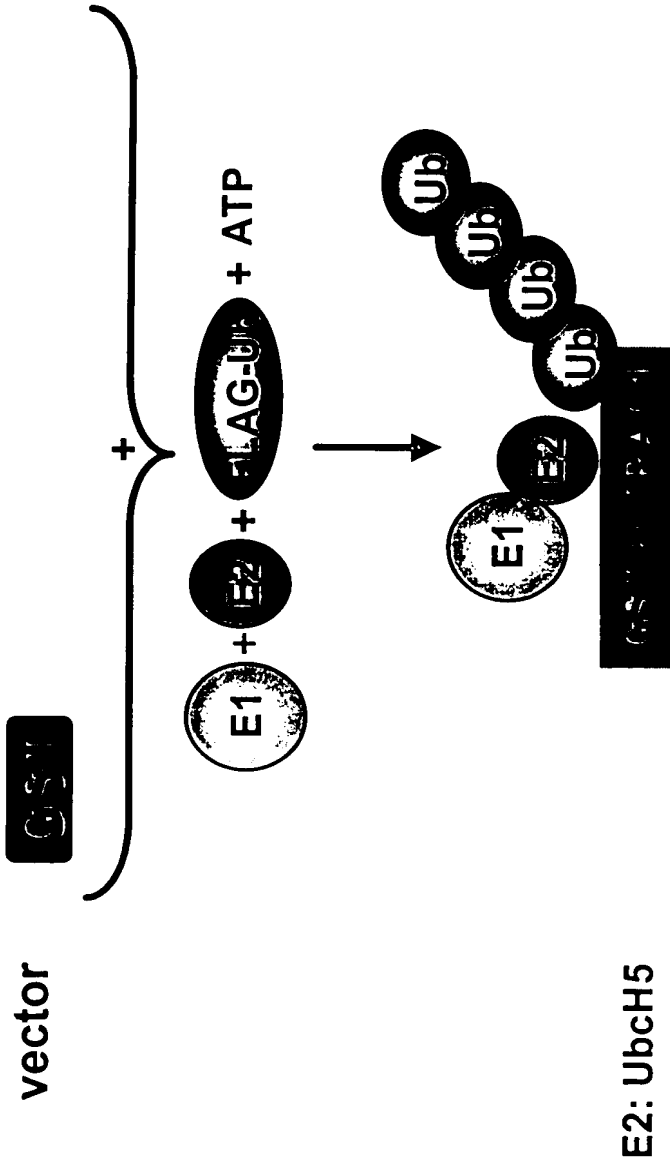- Proteasome-a 26S complex containing a 19S lid and base that mediates ATP- and ubiquitin-chain-dependent binding of substrates and a 20S catalytic core with three known proteolytic activities.
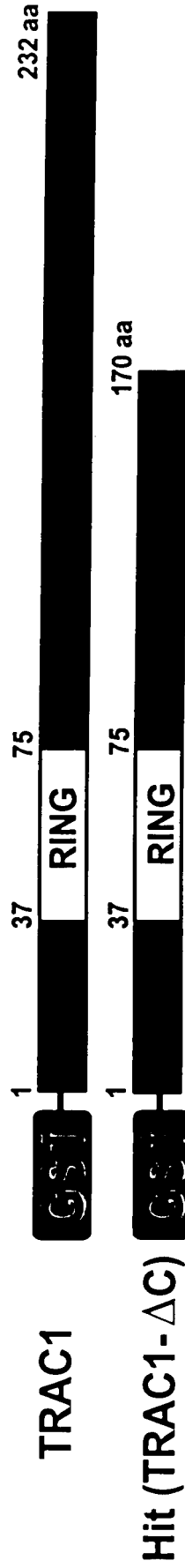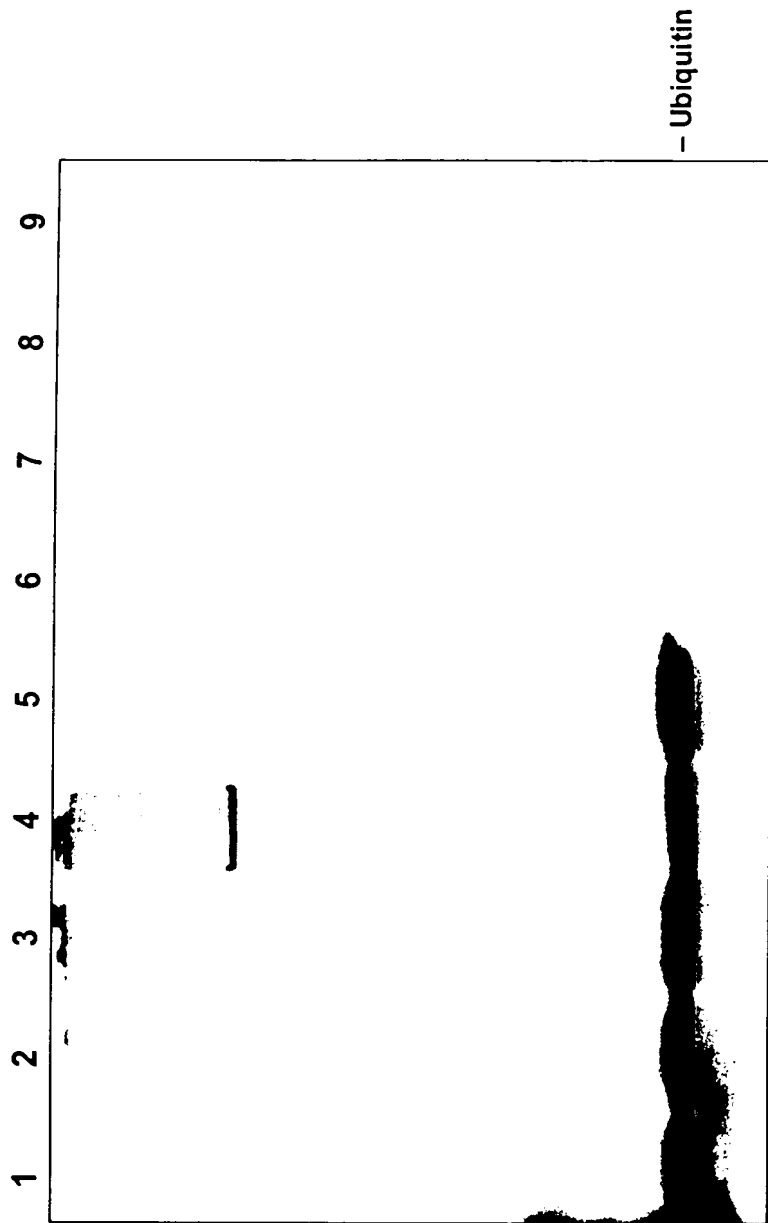
Figure 10B

# Enzymology of Ubiquitination

Figure 11A

# A Reconstituted, Substrate-independent Assay for Studying Ligase Catalysis

## Reaction Components

E1:

E2 (UbcH5): GST-fusion (cleaved), E.coli

E3 (Ring/cullin): His-tagged, coexpressed, baculovirus

Ubiquitin: FLAG-tagged, E.coli

Complete reaction

Reaction lacking:

E1  E2  E3  Ub  ATP

FLAG-$Ub_N$

FLAG-Ub

ATP

FLAG-Ub

E1  +

Roc1  Cul1  E2 — Catalytic core

→  Roc1  Cul1  E2  UbUbUbUbUbUb

The substrate-independent reaction has the same catalytic properties and requirements for Roc1/Cul1 as the substrate-dependent reaction

Figure 11B

Bacterially-expressed TRAC1 for Ligase Activity Assay

# TRAC1 Exhibits E3 Ubiquitin Ligase Activity

Figure 12A
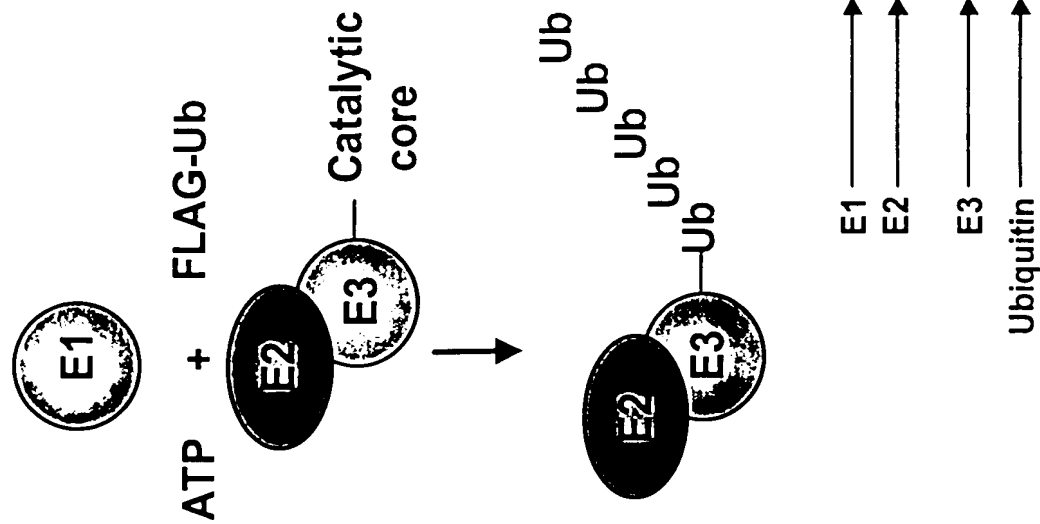
Figure 12B

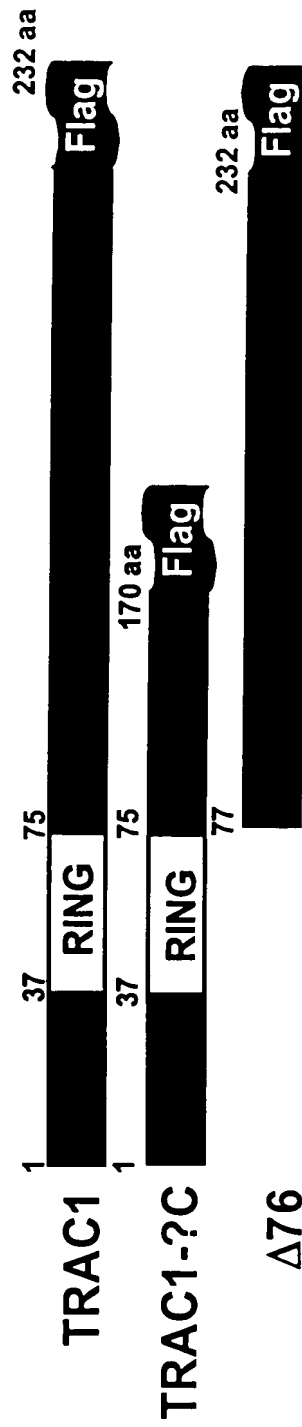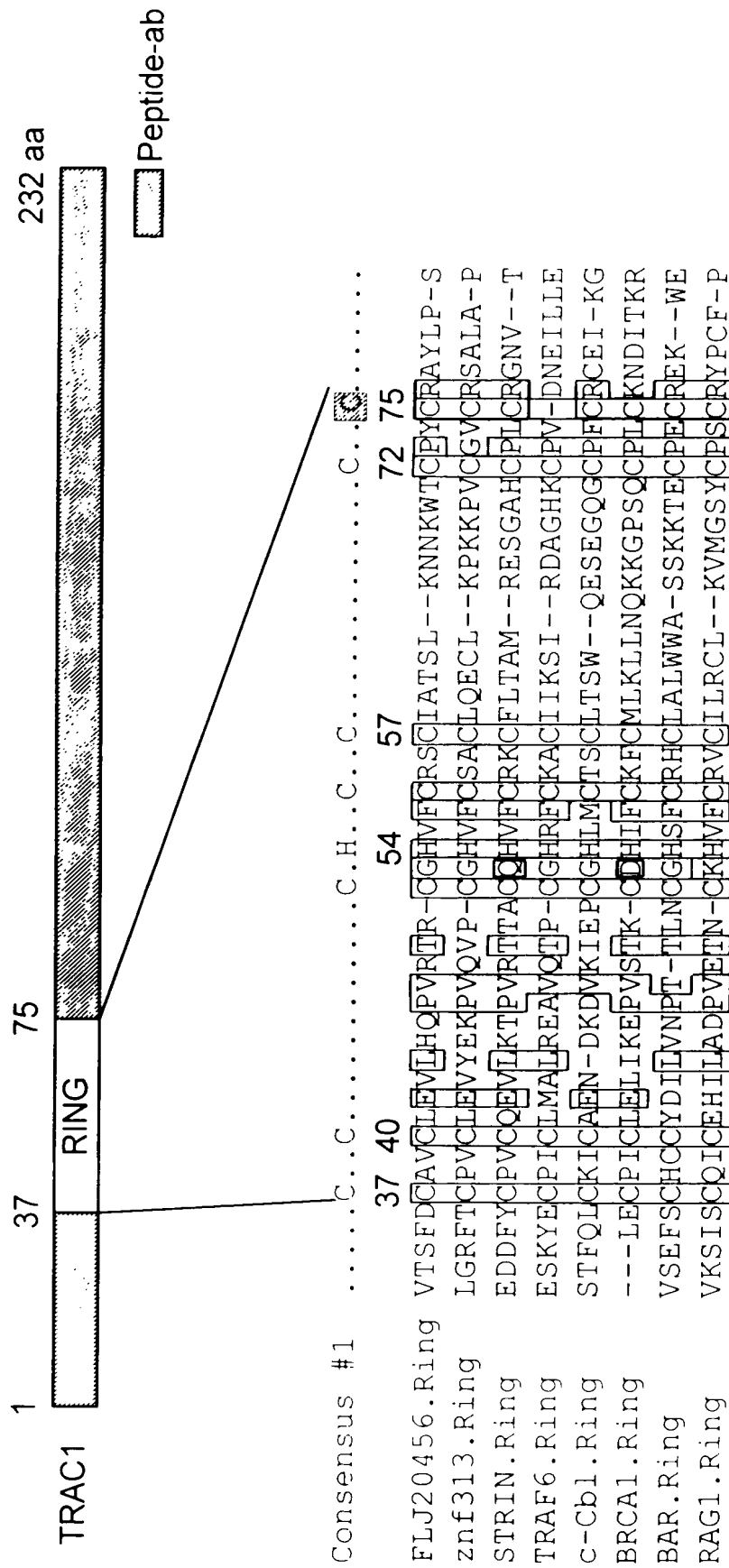The Ring Domain is Required for TRAC1 Ligase Activity
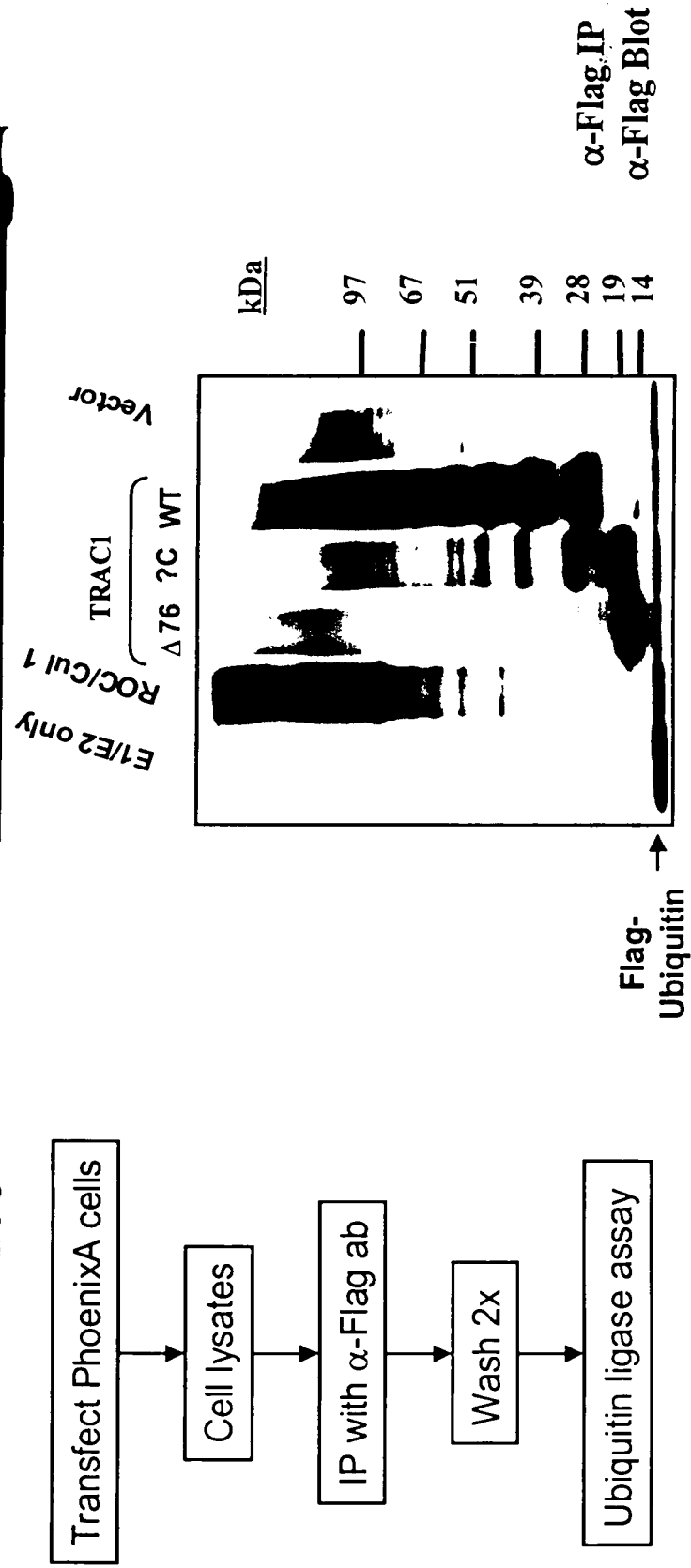
Point mutations in Conserved Cysteine Residues of the TRAC1 Ring finger Domain

TRAC1

| 1 | 37 | 75 | 232 aa |
|---|----|----|--------|

RING

☐ Peptide-ab

```
Consensus #1    ....C.C........C.H.C.C..........C
                   37 40        54 57       72 75

FLJ20456.Ring  VTSFDCAVCLEVLHQPVRTR-RSCIATSL--KNNKWTCPYCRAYLP-S
znf313.Ring    LGRFTCPVCLEVYEKPVQVP-CGHVFCSACLQECL-KPKKPVGVCRSALA-P
STRIN.Ring     EDDFYCPVCQEVLKTPVRTTAQGHVFCRKCFLTAM-RESGAHCPLCRGNV--T
TRAF6.Ring     ESKYECPICMALREAVQTP-CGHRFCKACIIKSI--RDAGHKCPV-DNEILLE
c-Cbl.Ring     STFQLCKICAEN-DKDVKIEPCGHLMCTSCLTSW--QESEGQGCPFCRCEI-KG
BRCA1.Ring     --LECPICLELIKEPVSTK-CDHIFCKFCMLKLLNQKKGPSQCPLCKNDITKR
BAR.Ring       VSEFSCHCCYDILVNPT-TLNCGHSFCRHCLALWWA-SSKKTECPECREK--WE
RAG1.Ring      VKSISCQICEHILADPVETN-CKHVFCRVCILRCL--KVMGSYCPSCRYPCF-P
```

• The following expression plasmids were generated:
pEFnig/Ring finger point mutants: H54A, C75A, C37, 40A, H54C57A, C72, 75A
pEFnig/Myristylation site mutant: G2A

*FIG. 13A.*

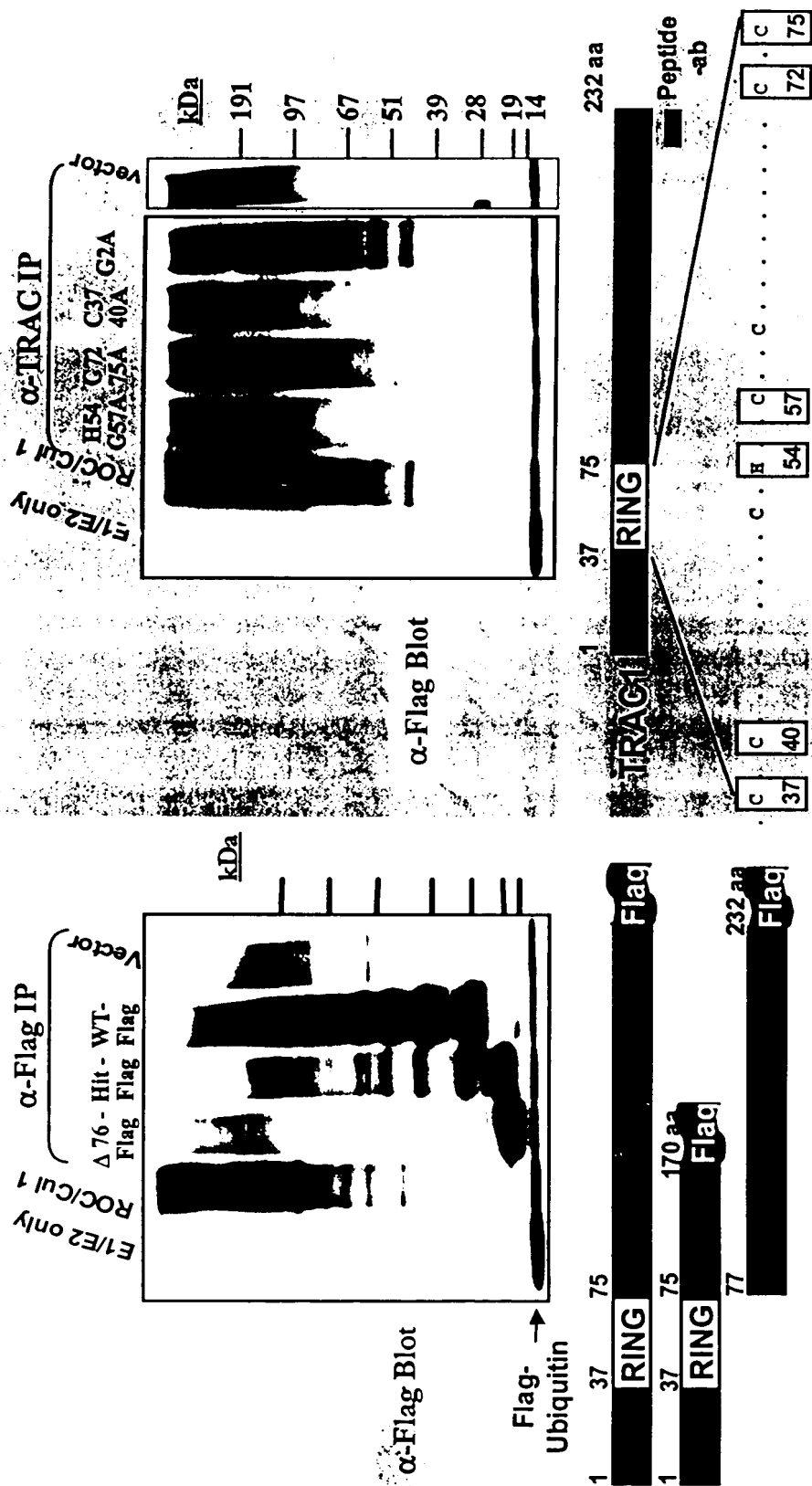# Point mutations in Conserved Residues of the TRAC1 Ring-finger Domain Disrupt Ligase Activity

Figure 14

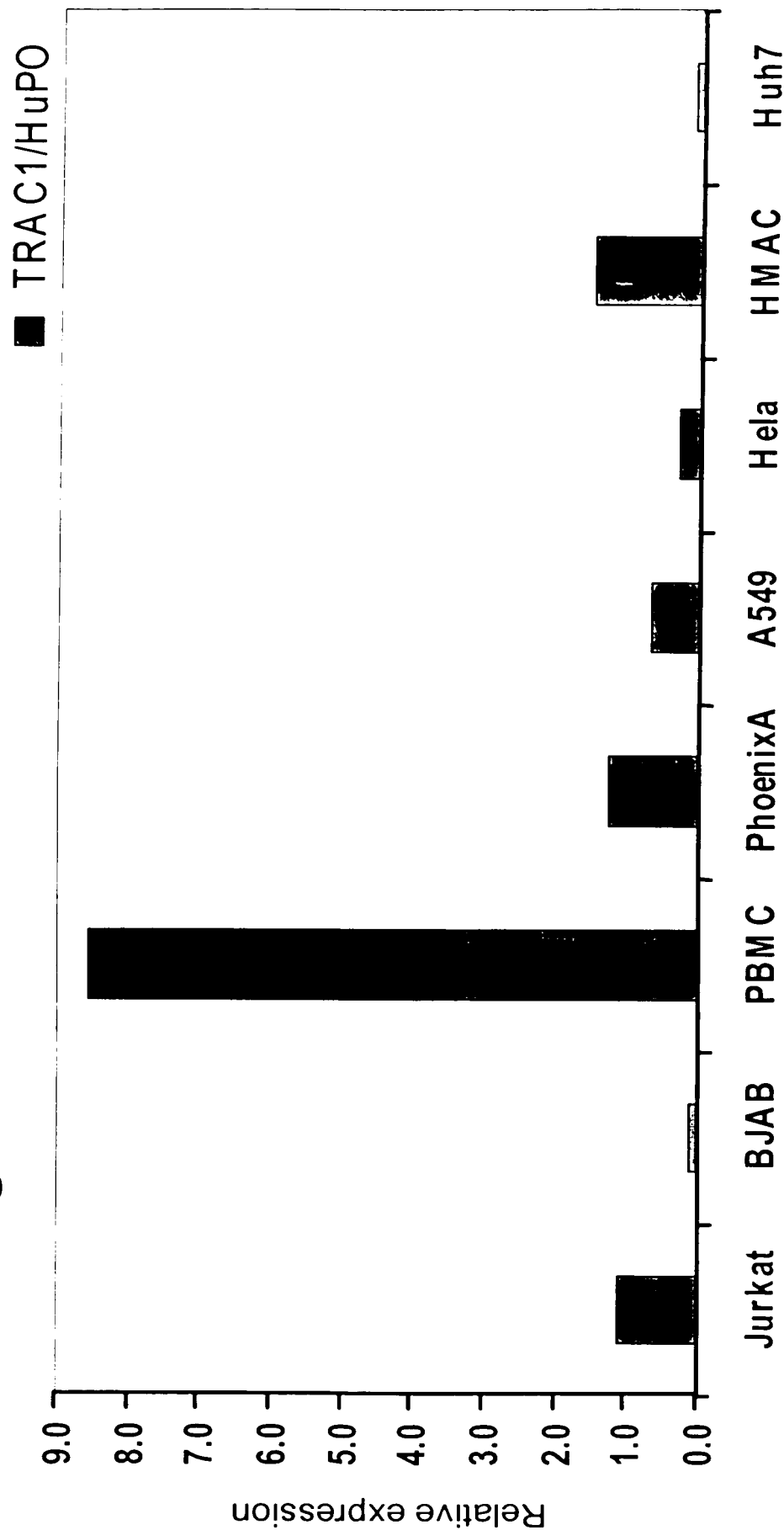# Expression of TRAC-1 mRNA is ~8 fold higher in PBMC than in Jurkat cells

Figure 15A

# C-terminal Truncated TRAC1 Blocks
# TCR-induced Ca2+ Influx
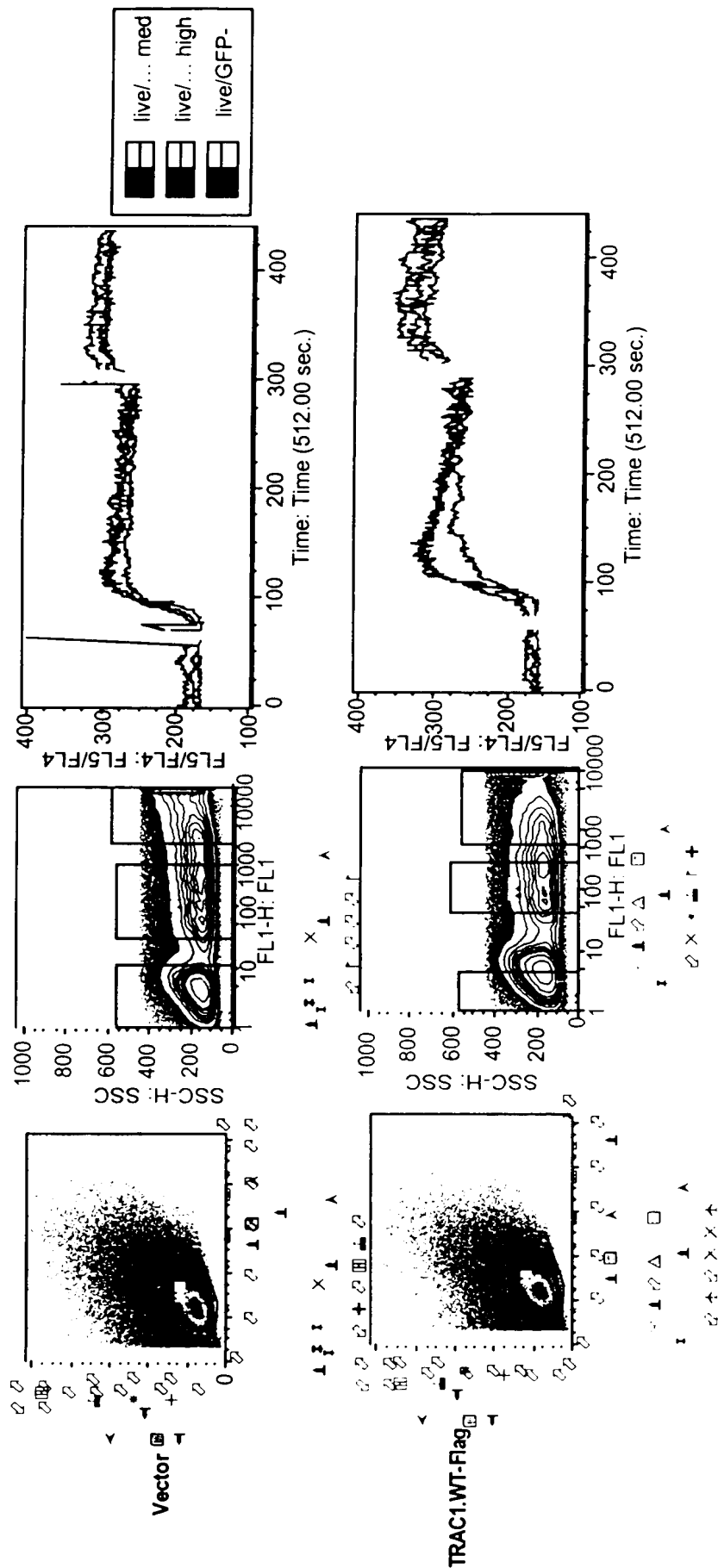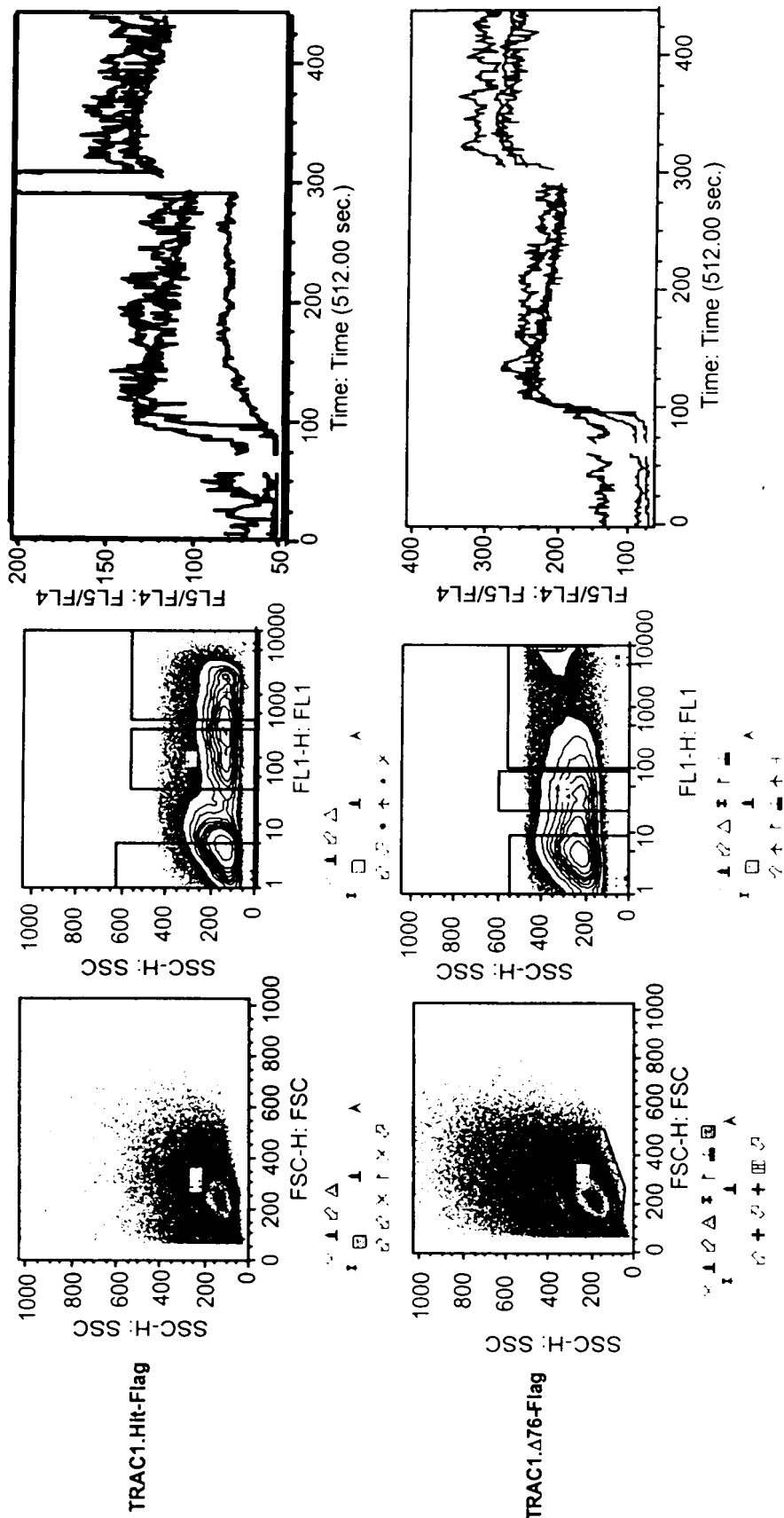
**Figure 15B**

Figure 16

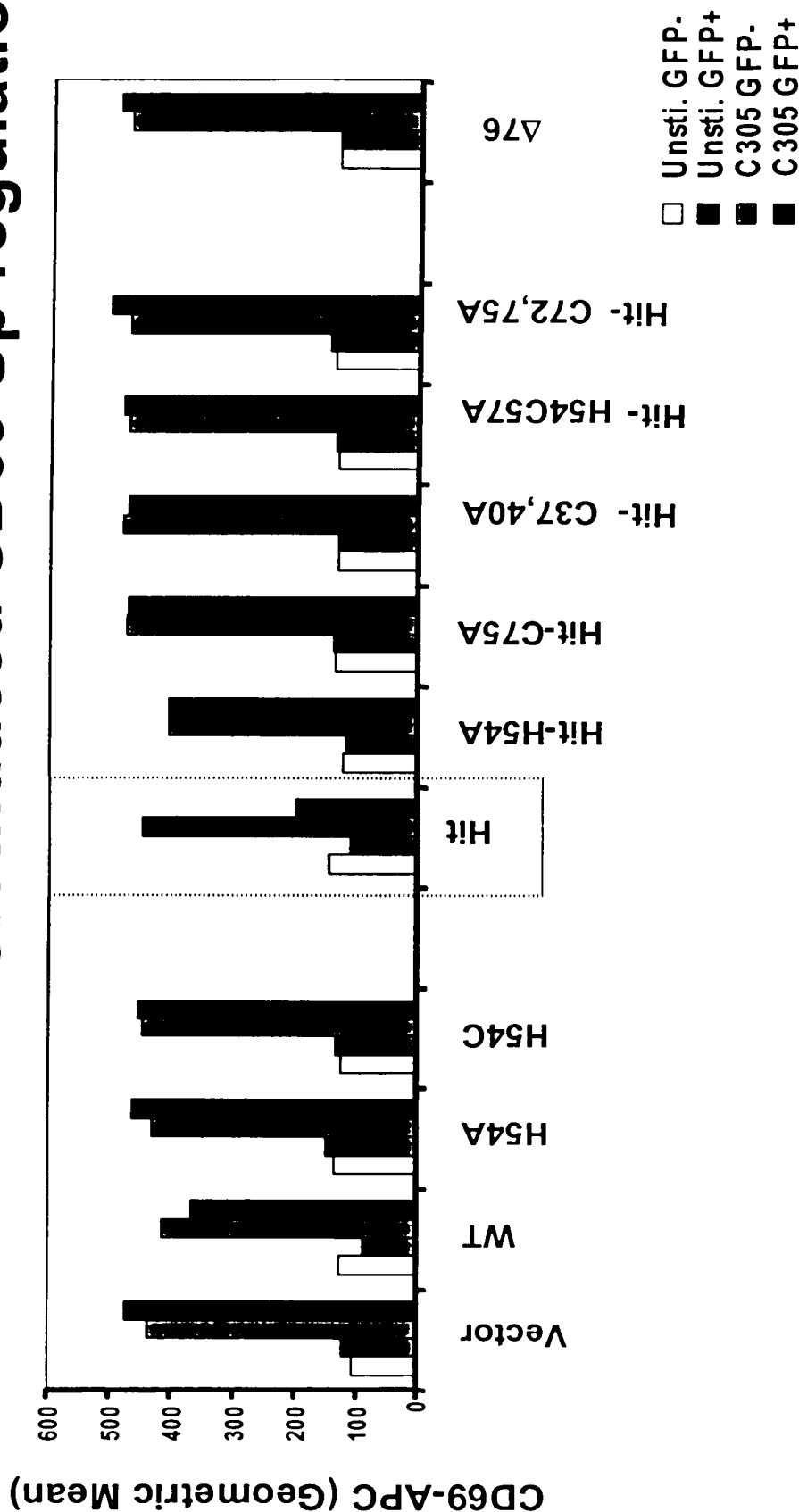# An Intact TRAC1 Ring domain is Required for Inhibition of α-TCR-Induced CD69 Up-regulation



Legend:
- □ Unsti. GFP-
- ■ Unsti. GFP+
- ■ C305 GFP-
- ■ C305 GFP+

Y-axis: CD69-APC (Geometric Mean), scale 0, 100, 200, 300, 400, 500, 600

X-axis categories: Vector, WT, H54A, H54C, Hit, Hit-H54A, Hit-C75A, Hit- C37,40A, Hit- H54C57A, Hit- C72,75A, Δ76

Figure 17

Summary of Functional Effects by Different TRAC-1 constructs

Figure 18

# Transiently Transfected TRAC1 Protein Binds to Ubiquitin - Conjugating Enzymes (E2s) UbcH7 and UbcH5 *in vitro*
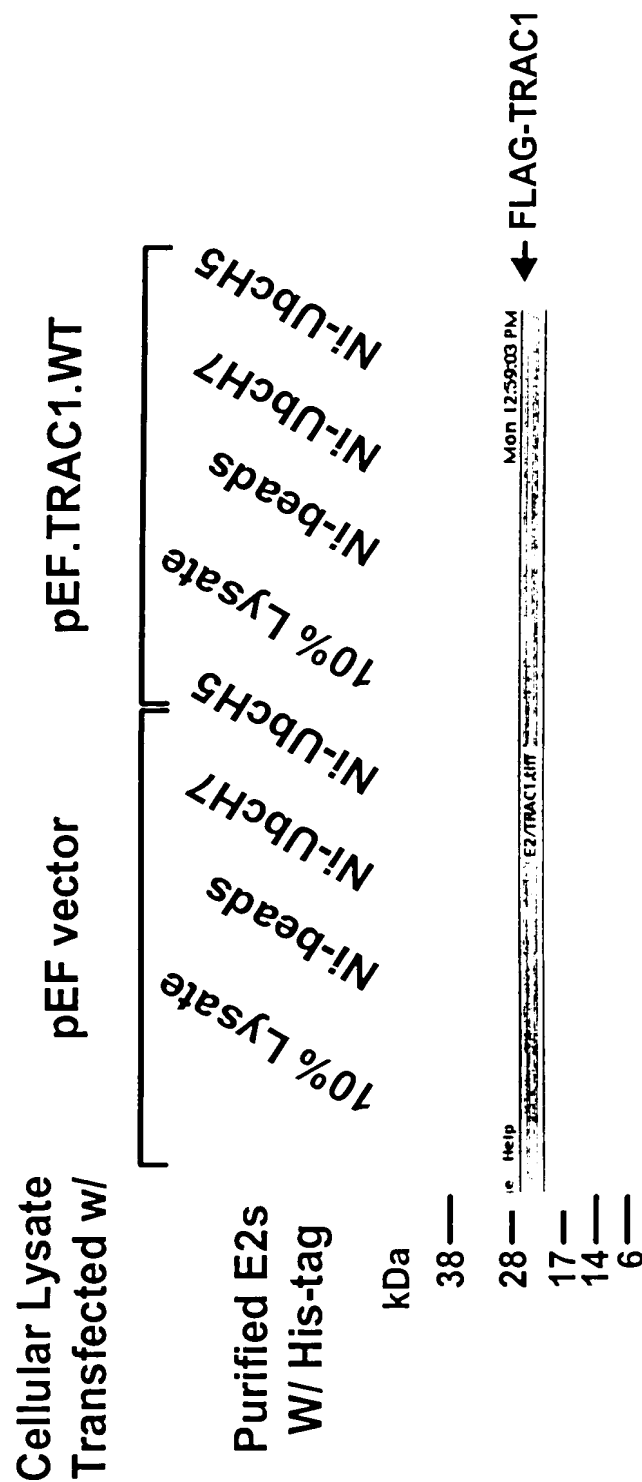
Figure 19

Model for TRAC-1 regulation of T cell activation